PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 501742/JEP	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/001310**	International Filing Date *(day/month/year)* 6 October 2003	Priority Date *(day/month/year)* 4 October 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] C07K 14/475		
Applicant COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheet(s).

3. This report contains indications relating to the following items:

I	[X]	Basis of the report
II	[ ]	Priority
III	[X]	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[X]	Certain defects in the international application
VIII	[X]	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
22 January 2004	28 January 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer O.L. CHAI Telephone No. (02) 6283 2482

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/001310

I. Basis of the report

1. With regard to the elements of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **1-13, 15-32, 34-54, and appendix 1, pages 1/67 - 67/67** as originally filed,
  pages , filed with the demand,
  pages **14, 33** received on **6 April 2004** with the letter of **5 April 2004**
- [X] the claims, pages **55-62** as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the drawings, pages **1/3 - 3/3** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [X] the sequence listing part of the description:
  pages **1/10 - 8/10** as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be nonobvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos: **40**

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. **40** are so unclear that no meaningful opinion could be formed *(specify)*:

Claim 40 includes matter which owes nothing to the teaching of the specification. The methods claimed in the antecedent claims may identify properties of existing compounds, but do not provide new compounds.

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claim Nos. **40**

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-14, 25-39, 41, 42	YES
	Claims	15-24, 43-46	NO
Inventive step (IS)	Claims	1-14, 25-39, 41, 42	YES
	Claims	15-24, 43-46	NO
Industrial applicability (IA)	Claims	1-39, 41-46	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Documents considered:

D1 F XU et al. International Journal of Cancer. Vol. 53, 1993, pp401-408.

D2 R M NEVE et al. Biochemical and Biophysical Research Communications. Vol. 280, 2001, pp274-279.

D3 F CENTIS et al. Hybridoma. Vol. 11, no. 3, 1992, pp 267-276.

D4 US 5968511 (AKITA et al). October 19, 1999.

D5 WO 1999/031140 (GENENTECH, INC). 24 June 1999.

D6 WO 2001/015730 (GENENTECH, INC). 8 March 2001.

D7 E ENAN et al. Journal of Biochemical and Molecular Toxicology. Vol. 12, no. 2, 1998, pp83-92.

D8 X LI et al. Cancer Gene Therapy. Vol. 8, no. 8, 2001, pp555-565.

D9 I STANCOVSKI et al. Proceedings of the National Academy of Science, USA. Vol. 88, 1991, pp8691-8695.

D10 T P J GARRETT et al. Molecular Cell. Vol. 11, 2003, pp495-505.

D11 Y L YIP et al. International Journal of Cancer. Vol. 104, 2003, pp303-309.

D12 J SINGH et al. Journal of Medicinal Chemistry. Vol. 40, 1997, pp1130-1135.

Novelty

Documents D1-D9 do not disclose a three-dimensional structure of ErbB2 corresponding to that disclosed in the present specification. Claims which comprise this feature are considered novel over the prior art.

Compounds binding to the ectodomain (ie., the N-terminal region) of ErbB2 are generally known in the art. Similarly, the use of such compounds to modulate the ligand-internalising properties of ErbB2 is generally known. Such compounds and uses do not rely on the provision of a crystal structure of the region 1-509 of ErbB2.

D1-D6, D8 and D9 disclose antibodies which bind various epitopes on the ErbB2 ectodomain and modulate its function. These documents anticipate claims 15-21, 24 and 43-46.

D7 discloses small molecules which bind to the ErbB2 ectodomain and modulate its function. This document anticipates claims 15-23 and 46.

Continued on Supplemental Sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

The specification does not conform to Article 3(2) of the PCT. The pages forming appendix 1 (1/67-67/67) should properly be part of the description or figures.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 32 and 34 do not appear to be supported by the description. The claims are not limited to the ErbB2 polypeptide crystals provided by the applicant in the specification or crystals reasonably derived therefrom. Only a single such crystal has been provided; given the nature of the technology, it does not appear reasonable that the conditions provided could be extrapolated to provide crystals of any given ErbB2 polypeptide.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Novelty, continued

D10-D12 are non-patent documents that were published after the priority date of the present application. These documents are presented for information only. D10 discloses the presently claimed truncated-ErbB2 crystal structure. D11 discloses a structural analysis of the ErbB2 receptor using antibodies. D12 discloses the structure-based design of an ErbB2 receptor inhibitor using a crystal structure of a cAMP dependent Ser/Thr kinase.

Inventive step

Further to the above considerations, it is considered that the subject matter of claims 15-24 and 43-46 is obvious in the light of D1-D9 cited above.

Industrial Applicability

The invention as claimed is industrially applicable.

entitled Current Protocols in Molecular Biology, which are incorporated herein by reference) and chemical methods.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

ErbB2 crystals and crystal structures

The present invention provides a crystal comprising an ErbB2 polypeptide. Such crystals preferably are of the space group $P2_12_12_1$ with unit cell dimensions of a=75.96 Å, b=82.24 Å, and c=110.06 Å.

As used herein, the term "crystal" means a structure (such as a three dimensional (3D) solid aggregate) in which the plane faces intersect at definite angles and in which there is a regular structure (such as internal structure) of the constituent chemical species. The term "crystal" refers in particular to a solid physical crystal form such as an experimentally prepared crystal.

Crystals according to the invention may be prepared using full-length ErbB2 polypeptides. However, preferably the extracellular domain is employed in isolation. Thus, preferably the ErbB2 polypeptide is a truncated polypeptide containing the extracellular domain and lacking the transmembrane domain and the intracellular tyrosine kinase domain. Typically, the extracellular domain comprises residues 1 to 632 (mature receptor numbering) of human ErbB2, or the equivalent thereof, or a truncated version thereof, preferably comprising amino acids 1 to 509, or the equivalent residues in other ErbB2 polypeptides.

In a preferred embodiment the ErbB2 polypeptide is human ErbB2 (Accession No. A24571 – mature protein begins at residue 22). However, the ErbB2 polypeptide may also be obtained from other species, such as other mammalian species.

crystallising the purified protein(s). Preferably the ErbB2 polypeptide contains the extracellular domain (amino acids 1 to 632 of the mature human polypeptide or a truncated version thereof, preferably comprising amino acids 1 to 509, or the equivalent residues in other ErbB2 polypeptides) but lacks the transmembrane and intracellular domains. Preferred host cells are those that provide for reduced glycosylation of recombinant polypeptides, such as a glycosylation-defective mammalian cell line e.g. the Lec8 Chinese hamster cell line, a derivative of CHO-K1 fibroblasts (ATCC CRC:1737) (Stanley, 1989, Mol. Cell Biol. 9: 377-383).

ErbB2 polypeptides may also be produced as fusion proteins, for example to aid in extraction and purification. Examples of fusion protein partners include glutathione-S-transferase (GST), hexahistidine, GAL4 (DNA binding and/or transcriptional activation domains) and beta-galactosidase. It may also be convenient to include a proteolytic cleavage site between the fusion protein partner and the protein sequence of interest to allow removal of fusion protein sequences.

After expression, the proteins may be purified and/or concentrated, for example by immobilised metal affinity chromatography, ion-exchange chromatography, and/or gel filtration.

The protein(s) may be crystallised using techniques described herein. Usually, in a crystallisation process, a crystallisation buffer is prepared with a lower concentration of a precipitating agent necessary for crystal formation. For crystal formation, the concentration of the precipitating agent has to be increased, by addition of precipitating agent or by diffusion of the precipitating agent between the crystallisation buffer and a reservoir buffer. Diffusion may be achieved by known techniques such as the "hanging drop" or the "sitting drop" method. In these methods, a drop of crystallisation buffer containing the protein (s) is hanging above or sitting beside a much larger pool of reservoir buffer. Alternatively, the balancing of the precipitating agent can be achieved through a semi-permeable membrane that separates the crystallisation buffer and prevents dilution of the protein into the reservoir buffer.

We have found that the inclusion of about 15% PEG 1500 provides optimal crystallization conditions for the extracellular domain of human ErbB2.